Marasmius androsaceus L.es Fr extract, piperidone derivative, and their use for the preparation of antihypertensives

Technical field

The present invention relates to Marasmius androsaceus L.es Fr extract, piperidone derivative, and methods for their preparation as well as use of the same for the preparation of antihypertensives.

Background art

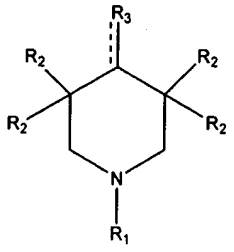
Hypertension and its related diseases are one kind of diseases seriously threaten human health and lives. Numerous peoples are suffering from it, and the patient tends to be younger in recent years. Although human has developed various drugs for the prevention and treatment of hypertension, it is still urgent to exploit new type of effective hypotensors.

Contents of the invention

The present inventors have been discovered by research that Marasmius androsaceus L.es Fr extract and piperidone derivative having the following formula I had very notable effect of reducing blood pressure, and thus could be used in the preparation of a medicament for prevention and/or treatment of hypertension and its related diseases.

Therefore, the first aspect of the present invention relates to Marasmius androsaceus L.es Fr extract, characterized by comprising 3,3,5,5-tetramethyl-4-piperidone, i.e., a compound of the following formula II.

In a second aspect the present invention provides a compound of the following formula I or a pharmaceutically acceptable salt thereof:



I

wherein,

R_1 and each of R_2, the same or different, independently represent hydrogen atom or C_1-C_5 alkyl;

⋯⋯ represents a single bond or a double bond, when ⋯⋯ is a double bond, R_3 is oxygen atom; when ⋯⋯ is a single bond, R_3 is hydroxyl group.

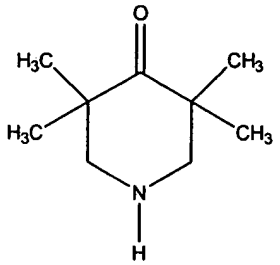
In a third aspect the present invention relates to a method for the preparation of Marasmius androsaceus L.es Fr extract, which comprises extracting fungi Marasmius androsaceus L.es Fr mycelium with an organic solvent, an aqueous organic solvent or water, and concentrating the resultant extractive to obtain the extract.

In a fourth aspect the present invention provides a method for the preparation of a compound of formula I, which comprises extracting, e.g., fungi Marasmius androsaceus L.es Fr mycelium with an organic solvent, an aqueous organic solvent or water to obtain an extract, separating and purifying the extract thereby obtaining a compound monomer of formula II, and then deriving the compound monomer by reacting it with a calculated amount of alkyl halide under basic condition.

In a fifth aspect the present invention relates to a pharmaceutical composition comprising as an active ingredient Marasmius androsaceus L.es Fr extract, or a compound of formula I, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers or excipients.

In a sixth aspect the present invention relates to use of Marasmius androsaceus L.es Fr extract or a compound of formula I in the preparation of a medicament for the prevention and/or treatment of hypertension and its related diseases.

In one preferred embodiment of the present invention, the compound of formula I is a compound of formula I wherein R_1 is hydrogen, each of R_2 is methyl, ⋯⋯ is a double bond and R_3 is oxygen atom, i.e., the compound of the following formula II:



II

Marasmius androsaceus L.es Fr is dried mycelium of Tricholomataceae fungi Marasmius androsaceus L.es Fr. It is a type of edible fungi, and also a common Chinese medicine, and has the effects of, e.g., relaxing and activating the tendons and retinervus, and reliving pain.

According to the present invention, Marasmius androsaceus L.es Fr extract can be prepared according to the following method:

Fungi Marasmius androsaceus L.es Fr bacteria, as purchased from Institute of Microorganism, Chinese Academy of Sciende, with bacterial number of 5.512

a. Fermentation culturing

A culture medium is prepared by mixing the following components (by weight): bran 5%, glucose 4%, corn steep liquor 0.4%, magnesium sulfate 0.05%, and potassium dihydrogen sulfate 0.1%. A slant strain is transferred to the culture medium, and cultured at 22-26°C for 7-8 days until forming sericate hypha and the culture medium becoming light yellow. The culture is stopped at pH = 3.5-4.0.

b. The fermentation broth as above cultured is filtered to obtain mycelium.

c. The mycelium is extracted with an organic solvent, an aqueous organic solvent or water. The obtained extractive is filtered and concentrated thereby obtaining Marasmius androsaceus L.es Fr extract.

According to the present invention, the piperidone derivative of formula I can be prepared, as above described, by the steps of: extracting, e.g., fungi Marasmius androsaceus L.es Fr mycelium with an organic solvent, an aqueous organic solvent or water to obtain an extract, separating and purifying the extract thereby obtaining a compound monomer of formula II, and then suitably deriving the obtained compound monomer.

Particularly, the method for preparing the piperidone derivative of formula I further comprises the following steps on the basis of the above steps a-c for preparing the Marasmius androsaceus L.es Fr extract:

d. The resultant extract is separated by chromatography with a silica gel column, and undergone gradient elution using chloroform:methanol = 1-100%; 80-90% of the chroroform fraction is collected to obtain a refined product of the compound of formula II; then, the resultant product is repetitively recrystallized with ethyl acetate/methanol thereby obtaining a compound monomer of formula II.

e. The compound of formula II is dissolved in a pH = 8-12 alkali methanol solution, to which a calculated amount of alkyl halide is added; the system undergoes reaction at 40-60°C for 10 h; then, the resultant reaction product is concentrated, and repetitively recrystallized thereby obtaining the compound of formula I.

In the step c of the above methods for preparing Marasmius androsaceus L.es Fr extract and the compound of formula I, the organic solvent used includes alcohols such as methanol, ethanol, propanol and butanol; alkyl halides such as dichloromethane and trichloromethane; esters such as methyl acetate, ethyl acetate and propyl acetate; and also ethers such as petroleum ether and diethyl ether. The preferred solvent is chloroform.

Pharmacological study shows that the extract of the present invention characterized by comprising the compound of formula II, the refined product thereof, i.e., the compound monomer of formula II, as well as the derived product of the compound of formula II, i.e., the compound of formula I all have very obvious effects of reducing blood pressure, which are mainly manifested themselves in that:

a. 50-100 mg/kg of the exact exhibits a very obvious effect of reducing blood pressure with respect to the models of anesthetic hypertensive rat and cat. 5-20 mg/kg of the compound of formula II exhibits a very obvious effect of reducing blood pressure with respect to the models of anesthetic rat and cat. 3-50 mg/kg of the compound of formula I exhibits a very obvious effect of reducing blood pressure with respect to the models of anesthetic rat and cat. All the effects of reducing blood pressure as above described can be maintained for above 4 h.

b. All the extract, the compound of formula II and the compound of formula I can inhibit

the automatic rhythmic contraction of ileum section in guinea pig, which all can slowly relax smooth muscle within a concentration range of 5×10^{-4} to 10×10^{-2} mg/ml, until a complete relaxation within 3 to 5 min. The relaxation time may be up to 4-5 h (flushing once per 15 min). Isoprenaline at a concentration of 5×10^{-5} mg/ml is used as a control, and the contraction inhibited thereby will be resumed in 20 min after flushing.

The above results show that the extract, the compound of formula II and the compound of formula I can inhibit the automatic rhythmic contraction of ileum section for an obviously longer time than isoprenaline as a control.

c. The extract, the compound of formula II and the compound of formula I all show significant effects on aorta smooth muscle of rabbit, which can inhibit the contraction of aorta smooth muscle of rabbit caused by adrenalin within a concentration range of 5×10^{-4} to 10×10^{-2} mg/ml, and will cause the relaxation of aorta smooth muscle of rabbit at a concentration of greater than 5×10^{-5} mg/ml.

The above results show that the extract, the compound of formula II and the compound of formula I have good effects of relaxing smooth muscle and excellent effects of reducing blood pressure, with hold time greater than 4 h.

Therefore, the Marasmius androsaceus L.es Fr extract and the compound of formula I can be used in the preparation of a medicament for the prevention and/or treatment of hypertension and its related diseases, said hypertension and its related diseases including simple hypertension and coronary heart diseases caused by hypertension as well as other cardio- and cerebro-vascular diseases.

According to the present invention, the pharmaceutically acceptable salt of the compound described herein includes acid-addition salts formed with inorganic or organic acids therewith or base-addition salts formed with bases therewith. Wherein, the acid-addition salts include, but are not limited to, hydrochlorides, hydrobromides, hydroiodides, nitrates, sulfates, bisulfates, phosphates, biphosphates, acetates, propionates, butyrates, trimethylacetates, adipates, alginates, lactates, citrates, tartrates, succinates, maleates, fumarates, picrates, aspartates, gluconates, benzoates, mesylates, ethylsulfonates, benzenesulfonates, p-toluenesulfonates and pamoates; and the base-addition salts include, but are not limited to, ammonium salts, alkali metal salts such

as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts; organic alkali salts such as dicyclohexyl amine and N-methyl-D-glucosamine salts, and amino acid salts such as arginine and lysine salts.

The Marasmius androsaceus L.es Fr extract or the compound of formula I of the present invention can be used alone, or in the form of a pharmaceutical composition by admixing it with a pharmaceutically acceptable carrier or excipient.

The pharmaceutical composition of the compound of the present invention can be administered by any of the following routes: oral, inhalation by spray, rectal, nasal, buccal, topical, parenteral such as injection or infusion by the routes of subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal and intracranial, or by the aid of an explanted container. In which, oral, injection or topical administration is preferred.

For oral administration, the compound of the present invention can be made into any of the dosage form suitable for oral administration, which include, but are not limited to, tablets, capsules, a solution or a suspension. Wherein, the carrier useful for tablets generally includes lactose and corn starch, to which a lubricant such as magnesium stearate may also be added. The diluent useful for capsule formulations generally includes lactose and dried corn starch. The aqueous suspension formulation is generally formed through mixing an active ingredient with suitable emulsifying agent and suspending agent. If desired, some sweetener, aromatizer or colorant may be further added to the above formulations for oral administration.

As to topical administration, in particular for the treatment of suffering surface or organ that is easily reached by topical application, such as the treatment of eyes, skin or lower enteric neurogenic diseases, the compound of the present invention can be made into different dosage form for topical administration according to different suffering surface or organ.

Particularly, for topical administration to the eyes, the compound of formula I of the present invention can be made into a dosage form of micronized suspension or solution, wherein the carrier used is isotonic sterile saline having a certain pH, to which an antiseptic such as chlorinated benzyl alkoxides may be added optionally. For ocular

administration, the compound may also be made into ointments such as vaseline ointments.

For topical administration to the skin, the compound of formula I of the present invention can be made into a dosage in the form of ointments, lotions or creams, wherein an active ingredient is suspended or dissolved in one or more carries. The carrier useful for the ointment formulations includes, but is not limited to, mineral oil, liquid vaseline, petrolatum album, propylene glycol, polyethylene oxide, polypropylene oxide, emulsified wax and water. The carrier useful for the lotion or cream formulations includes, but is not limited to, mineral oil, sorbitan monostearic esters, Tween 60, palmitates, hexadecylene aromatic alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compound of formula I of the present invention can also be administered in the form of sterile injection formulations, including sterile injection water or oil suspension or sterile injection solution. Wherein, the useable carrier and solvent include water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile nonvolatile oil, e.g., monoglyceride and diglyceride, can also be used as solvent or suspending medium.

Further, the administration dosage and manner of the compound described therein depend on various factors, such as age, body weight, gender, natural health status and nutrient status of the patient, activity of the compound, administration time, metabolic rate, severity of the disease, and subjective judgment made by the physician. The preferred administration dosage is within 0.01-100 mg/kg body weight/day, and the most preferred administration dosage is within 1-50 mg/kg body weight/day.

Specific mode of carrying out the invention

The following examples are for illustrative purposes only and do not intend to construct a limitation for this invention in any manner.

Example 1

Preparation of a chloroform extract

a. Culture of seeds of fungi Marasmius androsaceus L.es Fr:

A slant culture medium was prepared by mixing the following components (by weight):

bran 1-10, glucose 0.3-3, peptone 0.2-2, magnesium sulfate 0.01-0.1, potassium dihydrogen sulfate 0.02-0.2, agar 0.5-5 and water 100. Then, a strain was inoculated to the slant plane and cultured at 20-30°C for 10-20 days.

b. Fermentation culture of fungi Marasmius androsaceus L.es Fr:

A culture medium was prepared by mixing the following components (by weight): bran 3-30%, glucose 1-10%, corn slurry liquid 0.2-20%, magnesium sulfate 0.01-8%, and potassium dihydrogen sulfate 0.05-9%. The slant strain is transferred to the fermentation culture medium, and cultured by fermentation at 20-30°C for 5-10 days until forming sericate hypha, and the culture medium becoming faint yellow. The culture was stopped at pH = 1.5-6.0, and then the fermentation broth is allowed to stand by for 5-10 days.

c. The fermentation broth as above obtained was filtered to obtain mycelium. After crushing, 1,000 g of the mycelium passed through a sieve of 30-40 meshes, and then was extracted with 3-5 folds of water for 5 times. The obtained extractive was recovered at low temperature and reduced pressure. The residue was dissolved with 10% NaOH, and then extracted with 3-5 folds of chloroform for 5 times. After recovering chloroform, 3 g of chloroform extract was obtained.

d. The chloroform extract was in deep brown, insoluble in water, and easily soluble in organic solvent such as chloroform, ethanol and acetone, and exhibited positive in alkaloid reaction and for phenol sulfate. By TLC detection using chloroform:methanol:ammonia water = 9:1:0.1 as developing agent, five spots were observed, which R_f values were respectively 0.12, 0.23, 0.45, 0.56 and 0.71, wherein the substance corresponding to the spot of R_f = 0.56 was just the compound of formula II of the present invention.

Example 2

Preparation of a compound of formula II(3,3,5,5-tetramethyl-4-piperidone)

The chloroform extract obtained in step c of Example 1 was separated with a silica gel column using chloroform:methanol:ammonia water = 9:1:0.1 as developing agent. The eluents containing the compound of formula II were combined and concentrated to obtain a compound monomer of formula II.

The compound of formula II was a white needle crystal, with a melting point of 54-57°C (decomposition).

Elemental analysis□$C_9H_{17}NO$

	C(%)	H(%)	O(%)
Analysis values	69.48	10.96	10.12
Calculated values	69.68	10.97	10.32

MS(+FAB)m/z: 156.2, 149.2, 102.2, 98.2, 83.1, 74.0, by which the compound was verified as having a molecular weight of 155.

IR(KBr)cm^{-1}: 3318.93, 2910.09, 2755.81, 1727.93, 1626.67, 1726, 170, 1727.93, 1626.67, 1726.23, 2317.05, 2997-2465.

UV λ^{MeOH}_{MAX}nm□264.3 (ε13256).

^{1}H-NMR□DMSO□TMS□δppm□1.48(S,12H,$(CH_3)_4$)□2.63(S,4H,$(CH_2)_2$) 9.67(S,1H,NH).

^{13}C-NMR (DMSO-D_6,TMS) δppm: 27.20($CH_3)_4$, 49.79($CH_2)_2$, 59.09(C), 204.35(C=O).

^{1}H-^{13}C HMQC □ ^{1}H-^{13}C HMBC (long distance correlative) and data ascribed thereto were listed in Table 1.

Table1. Data of the compound of formula II as determined at 400 MHz

Position of C	δH(J in Hz ppm)	δC(ppm)	HMQC	HMBC
1	9.67(S,1H,NH)			
2	2.63(S,2H,CH_2)	59.09(C)	Correlative with H at 2-position	Correlative with H at 3'-position
3		49.79(CH_2)$_2$		Correlative with H at 3'-position Correlative with H at 2-position
3'	1.48(S,6H,(CH_3)$_2$)	27.20(CH_3)$_2$	Correlative with H at 3'-position	Correlative with H at 3'-position Correlative with H at 2-position
4		204.35(C=O)		Correlative with H at 3'-position Correlative with H at 2-position
5		49.79(CH_2)$_2$		
5'	1.48(S,6H,(CH_3)$_2$)	27.20(CH_3)$_2$		Correlative with H at 5'-position Correlative with H at 6-position
6	2.63(S,2H,CH_2)	59.09(C)		Correlative with H at 5'-position

Example 3

Preparation of 1-ethyl-3,3,5,5-tetramethyl-4-piperidone (compound of formula Ia)

0.3 g (1.94 mmol) of the compound of formula II obtained in Example 2 and 7.5 mmol

of bromoethane were dissolved with 40 mol of anhydrous ethanol. The solution was charged to a 100 ml three-neck flask equipped with a reflux condenser, a stirrer, an internal thermometer and a dropping funnel. An ethanol solution containing 8.5 mmol sodium ethoxide was added to the flask with stirring followed by reacting the system at 50°C for 20-50 min. After cooling down, 20 ml of chloroform was dropped to the system before standing by for a certain time. The resultant product was filtered to remove sodium bromide, and the filtrate was concentrated to dry under vacuum condition. Then, the reaction product was separated with a silica gel column, and eluted using chloroform:methanol (5:1), thereby obtaining the titled compound of formula Ia: 1-ethyl-3,3,5,5-tetramethyl-4-piperidone.

FAB-MS m/z: 185$[M+H]^+$, by which the compound was verified as having a molecular weight of 184.

Example 4

Preparation of 3,3,5,5-tetramethyl-4-piperidanol (compound of formula Ib)

0.5 g (3.22 mmol) of the compound of formula II obtained in Example 2 was dissolved with 50 ml chloroform. The obtained solution was charged to a three-neck flask equipped with a reflux condenser, a stirrer and an internal thermometer. Sodium borohydride as a reducing agent was added in an equal mole to the solution before allowing the system to react at 50°C for 100-120 min. The resultant product was filtered to remove the reducing agent, and the filtrate was concentrated to dry under vacuum condition. Then, the reaction product was separated with a silica gel column, and eluted using chloroform:methanol (5:1), thereby obtaining the titled compound of formula Ib: 3,3,5,5-tetramethyl-4-piperidanol.

FAB-MS m/z: 158$[M+H]^+$, by which the compound was verified as having a molecular weight of 157.

Example 5

Preparation of a hydrochloric acid salt of the compound of formula II

1 g (6.45 mmol) of the compound of formula II obtained in Example 2 was charged to a 300 ml three-neck round flask equipped with a reflux condenser, a stirrer, an internal thermometer and a dropping funnel. In a water bath of 80°C, the compound was completely dissolved by adding 100 ml acetone with stirring and thereafter a 6N HCl solution in an equal mole was slowly dropped to the solution. After finishing the dropping, the system is allowed to stand by for 10 min before recovering acetone till dry. The residue was dissolved with 30 ml chloroform followed by filtering, and then a 10 ml ethyl acetate solution was added to the filtrate. After standing by for 5 h, the solution was filtered and crystallized, thereby obtaining the titled hydrochloric acid salt of the compound of formula II: 3,3,5,5-tetramethyl-4-piperidone hydrochloride.

Example 6

Effects of the chloroform extract, the compound of formula II and the compound of formula Ib on ileum smooth muscle of guinea pig

About 0.5 g of the chloroform extract, the compound of formula II or the compound of formula Ib was exactly weighted, and dissolved with distilled water to obtain a sample solution of about 10 mg/ml. An ileum of guinea pig is collected and is flushed with a pre-cooled Tai's nutrient solution (which comprised of 1000 ml water, 8 g NaCl, 0.2 g KCl, 0.1 g $MgCl_2$, 0.05 g NaH_2PO_4, 1 g $NaHCO_3$, 0.2 g $CaCl_2$ and 1 g glucose with pH = 7.4) to remove food residue therein, and then said ileum is cut into a section of 3 cm long. The section was clamped at its both ends with frog heart clips, and placed in a perfusion tank with the lower end being fixed at the bottom of the tank and the top end being connected by a thread to a tensile transducer of a two-way electrophysiograph, so that the automatic rhythmic contraction of the ileum section was recorded. The Tai's nutrient solution in the tank was kept at a temperature of 35°C, into which pure nitrogen was bubbled. The ileum section was exerted with 1 g of pulling force when being fixed, and its contraction was recorded after balancing for 40 min, during which the Tai's nutrient solution was changed once per 20 min. The normal contraction curve of the ileum section was firstly recorded using isoprenaline as a positive control. Then, the

contraction curves of the ileum section were recorded after adding the chloroform extract, the compound of formula II or the compound of formula Ib of different concentrations respectively. The results were shown in Table 2.

Table 2. Effects of different samples on ileum smooth muscle of guinea pig

Name of Sample	Concentration □mg/ml□	Time of taking effect (min)	Relaxation Time(h)
Isoprenaline	5×10^{-5}	16	0.3
Chloroform extract	5×10^{-3}	3	5
Compound of formula II	1×10^{-4}	2	7
Compound of formula Ib	1×10^{-4}	3	5

The experimental results showed that: all the chloroform extract, the compound of formula II and the compound of formula Ib of different concentrations could inhibit the automatic rhythmic contraction of the ileum section. The chloroform extract at a final concentration of 5×10^{-3} as well as the compound of formula II or the compound of formula Ib at a concentration of 1×10^{-4} could slowly relax the smooth muscle. The contraction of the smooth muscle could completely disappear after 2-3 min, and the relaxation of the smooth muscle could continue for 5-7 h (flushing once per 15 min). In contrast, the contraction of the smooth muscle inhibited by isoprenaline as a positive control at a concentration of 5×10^{-5} mg/ml was resumed in 20 min after flushing. The result indicates that the chloroform extract, the compound of formula II and the compound of formula Ib could take effect for a far longer time than the positive control.

Example 7

Effects of the chloroform extract, the compound of formula II and the compound of formula Ia on aorta of rabbit

A New Zealand rabbit was beheaded and u nconscious. After thoracotomy, its thoracic aorta was quickly taken out, and quickly placed in and flushed with a pre-cooled LOCK's solution (which comprised of 1000 ml water, 9 g NaCl, 0.35 g KCl, 0.35 g

$MgSO_4.7H_2O$, 0.16 g KH_2PO_4, and 1 g $NaHCO_3$) to remove bloodiness. The connective tissue outside the blood vessel was carefully cut off, and an aorta section of 2-3 mm wide at an angle of 45° relative to the vessel was taken for use in the following test.

The aorta section of 2 cm long was clamped at its both ends with frog heart clips, and put in a thermostatic perfusion tank of 38°C with the lower end being fixed at the bottom of the tank and the top end being connected by a thread to a tensile transducer of a two-way electrophysiolograph. Oxygen was bubbled to the solution in the perfusion tank, and the contraction force of the aorta section was recorded after balancing it in the solution for 80 min. During which, the LOCK's solution was changed once per 20 min. After adding 1.2 x 10^{-6} mg/ml adrenalin to the solution, the contraction curve of the aorta section was recorded. When the contraction height did not ascend anymore, the aorta section was flushed for 4 times. After 40 min, when the contraction of the aorta section was resumed, the chloroform extract, the compound of formula II or the compound of formula Ia of different concentrations was added and took effect for 10 min, followed by adding adrenalin at a concentration of 1.2 x 10^{-6} mg/ml. It was observed that adrenalin added here could resume the contraction force of the aorta section only to 1/2-1/3 of the original value. The results were listed in Table 3.

Table 3. Effects of different samples on aorta of rabbit

Name of Sample	Concentration (mg/ml)	Time of taking effect (min)	Contraction force
Isoprenaline	1.2×10^{-6}	16	1
chloroform extract	5×10^{-3}	3	0.5
compound of formula II	1×10^{-4}	2	0.3
compound of formula Ia	1×10^{-4}	3	0.3

The results demonstrated that the chloroform extract, the compound of formula II or the compound of formula Ia within a concentration range of 5 x 10^{-3} -1 x 10^{-4} mg/ml could obviously reduce the contraction of aorta section caused by adrenalin.

Example 8

Effects of the chloroform extract, the compound of formula II, the hydrochloric acid salt of the compound of formula II as well as the compound of formula Ib on the reduction of blood pressure of rat

A spontaneously hypertensive rat (SHR) was anesthetized with 40 mg/ml pentobarbital, and fixed by its back on an experimental stand. The skin at its neck was cut open after removing hair, and thereafter the right common carotid artery was separated with its proximal end being clamped by a artery clamp and its axifugal end being ligated by a thread. A V-shape cut was made with scissors at the axifugal end, into which an arterial cannula filled with heparin physiological saline was inserted. The arterial cannula was connected with a blood pressure transducer, and then the blood pressure transducer was connected to a computer-controlled three-way physiological-pharmacological recorder. The right femoral vein of the rat was separated and intubated for the injection of a drug. When the blood pressure became stable (i.e., the blood pressure contraction curve became straight), the rat was administered by intravenous route. The results were shown in Table 4.

Table 4. Effects of the chloroform extract, the compound of formula II and the compound of formula Ib on the reduction of blood pressure of SHR rat

Drug	Dosage (mg/kg)	Blood pressure (mm/Hg)	Before administration	Time after administration (min) 5	60	120	240
Chloroform extract	200	Systolic pressure	179±10	145±12*	124±10**	125±9.6**	162±12**
		Diastolic pressure	129±16	107±13*	88±1**	87±13**	113±10**
Compound of formula II	10	Systolic pressure	174±11	141±9.2*	120±12	125±14**	163±12**
		Diastolic pressure	124±12	105±11*	84±11**	83±21**	110±18**
Hydrochloric acid salt of compound of formula II	10	Systolic pressure	170±9.3	139±19*	123±11	129±17**	169±14**
		Diastolic pressure	119±15	109±13*	89±12**	80±23**	112±16**
Compound of formula Ib	10	Systolic pressure	178±13	140±19*	121±15**	126±16**	162±11**
		Diastolic pressure	125±17	1099±1*	88±10**	89±8.9**	107±9.2**

*□P<0.05 in comparison with that before administration.

**□P<0.01 in comparison with that before administration.

The experimental results demonstrated that all the chloroform extract, the compound of formula II, the hydrochloric acid salt of the compound of formula II as well as the compound of formula Ib within a dosage range of 10-200 mg/kg could obviously reduce the systolic pressure and diastolic pressure of SHR rat.

Example 9

Effects of the chloroform extract, the compound of formula I and the compound of formula Ib on the reduction of blood pressure of cat

Five cats of 2.5-3.2 kg were administered orally and intravenously respectively

according to the method in Example 8, and then their systolic pressure and diastolic pressure were determined using normal cats non-administered as a contol group. The results showed that all the compounds have very notable effects on the reduction of blood pressure (see Tables 5-9).

Table 5. Blood pressure values of cats as a control group

Sample, Time	Animal 1 Systolic pressure (mmHg)	Animal 2 Systolic pressure (mmHg)	Animal 3 Systolic pressure (mmHg)	Animal 4 Systolic pressure (mmHg)	Animal 5 Systolic pressure (mmHg)	Average Systolic pressure (mmHg)	Standard derivation	T value	P value
Control group Before administration	168	212	200	182	184	189.2	17.06458		
10 min after administration	168	212	195	183	187	189	16.17096	0.985289	
20 min after administration	162	206	195	184	187	186.8	16.2696	0.825656	
30 min after administration	168	212	203	180	173	187.2	19.27952	0.866419	
40 min after administration	162	203	215	179	173	186.4	21.92715	0.827366	
50 min after administration	160	190	215	172	173	182	21.31901	0.571749	
60 min after administration	160	210	190	172	178	182	19.0263	0.546305	
	Animal 1 Diastolic pressure (mmHg)	Animal 2 Diastolic pressure (mmHg)	Animal 3 Diastolic pressure (mmHg)	Animal 4 Diastolic pressure (mmHg)	Animal 5 Diastolic pressure (mmHg)	Average Diastolic pressure (mmHg)	Standard derivation	T value	P value
Before administration	144	131	125	148	156	140.8	12.63725		
10 min after administration	136	136	125	150	156	140.6	12.36123	0.980437	
20 min after administration	137	136	125	150	153	140.2	11.38859	0.939077	
30 min after administration	133	120	125	148	156	136.4	15.24139	0.632606	
40 min after administration	133	120	125	153	160	138.2	17.51285	0.794587	
50 min after administration	135	120	120	153	155	136.6	17.03819	0.669695	
60 min after administration	136	124	120	148	160	137.6	16.63731	0.74081	

Table 6. Effect of the chloroform extract (oral administration) on the reduction of blood pressure of cats

Sample, Time	Animal 1 Systolic pressure (mmHg)	Animal 2 Systolic pressure (mmHg)	Animal 3 Systolic pressure (mmHg)	Animal 4 Systolic pressure (mmHg)	Average Systolic pressure (mmHg)	Standard derivation	T value	P value
Chloroform extract 100 mg/kg o.s								
Before administration	200	205	216	183	201	13.7356		0.300196
10 min after administration	193	190	206	176	191.25	12.31192	0.331138	0.825362
20 min after administration	173	207	190	161	182.75	20.07278	0.184113	0.74712
30 min after administration	143	181	176	153	163.25	18.19112	0.01616	0.099648
40 min after administration	129	162	170	132	148.25	20.79062	0.005477	0.032864
50 min after administration	127	162	171	133	148.25	21.53099	0.006141	0.051095
60 min after administration	126	161	176	135	149.5	23.07235	0.0086	0.053166
90 min after administration	127	175	183	136	155.25	27.86126	0.025759	
120 min after administration	145	175	184	146	162.5	19.97498	0.019165	
150 min after administration	188	183	186	171	182	7.615773	0.051897	
180 min after administration	196	201	206	179	195.5	11.73314	0.564903	
	Animal 1 Diastolic	Animal 2 Diastolic	Animal 3 Diastolic	Animal 4 Diastolic	Average Diastolic	Standard derivation	T value	P value

	pressure (mmHg)	pressure (mmHg)	pressure (mmHg)	pressure (mmHg)	pressure (mmHg)			
Before administration	138	135	141	138	138	2.44949		0.679493
10 min after administration	130	140	157	130	139.25	12.73774	0.853524	0.876878
20 min after administration	115	140	137	125	129.25	11.5	0.187228	0.196542
30 min after administration	91	139	126	115	117.75	20.35313	0.095604	0.158499
40 min after administration	83	114	120	98	103.75	16.66083	0.006593	0.020113
50 min after administration	88	114	117	97	104	13.83233	0.002879	0.01765
60 min after administration	85	120	116	97	104.5	16.42153	0.006839	0.020448
90 min after administration	93	122	122	98	108.75	15.43535	0.009587	
120 min after administration	109	122	134	116	120.25	10.59481	0.01715	
150 min after administration	145	135	134	134	137	5.354126	0.74567	
180 min after administration	151	143	142	135	142.75	6.551081	0.223219	

Table 7 Effects of the compound of formula II (oral administration) on the reduction of blood pressure of cats

Sample, time	Animal 1 Systolic pressure (mmHg)	Animal 2 Systolic pressure (mmHg)	Animal 3 Systolic pressure (mmHg)	Animal 4 Systolic pressure (mmHg)	Average Systolic pressure (mmHg)	Standard derivation	T value	P value
Compound of formula II 10 mg/kg o.s								
Before administration	191	215	196	208	202.5	10.96966		0.221212
10 min after administration	190	180	159	136	166.25	23.9496	0.033194	0.131804
20 min after administration	170	181	138	123	153	27.06782	0.014681	0.052155
30 min after administration	154	140	138	130	140.5	9.983319	0.000159	0.00332
40 min after administration	146	133	143	140	140.5	5.567764	5.54E-05	0.004985
50 min after administration	149	140	144	144	144.25	3.685557	5.57E-05	0.010642
60 min after administration	149	153	130	144	144	10.03328	0.000223	0.008944
90 min after administration	154	180	154	140	157	16.69331	0.003868	
120 min after administration	146	195	163	156	165	21.18175	0.019962	
150 min after administration	146	206	168	157	169.25	26.09438	0.057113	
180 min after administration	158	213	170	164	176.25	24.985	0.102697	
210 min after administration	159	213	170	164	176.5	24.74537	0.103109	
240 min after administration	178	213	176	164	182.75	21.09305	0.147696	

	Animal 1 Diastolic pressure (mmHg)	Animal 1 Diastolic pressure (mmHg)	Animal 1 Diastolic pressure (mmHg)	Animal 1 Diastolic pressure (mmHg)	Average Diastolic pressure (mmHg)	Standard derivation	T value	P value
Before administration	143	165	145	137	147.5	12.15182		0.448148
10 min after administration	143	145	112	76	119	32.4037	0.150646	0.207389
20 min after administration	130	136	91	72	107.25	30.82613	0.051199	0.060186
30 min after administration	110	108	91	75	96	16.39105	0.002338	0.006499
40 min after administration	107	100	95	79	95.25	11.89888	0.000851	0.004198
50 min after administration	110	116	95	83	101	14.89966	0.00289	0.013399
60 min after administration	110	117	102	83	103	14.67424	0.003427	0.013881
90 min after administration	102	120	97	96	103.75	11.14675	0.001819	
120 min after administration	93	154	96	90	108.25	30.5982	0.054443	
150 min after administration	93	161	109	84	111.75	34.42262	0.097877	
180 min after administration	107	164	112	87	117.5	32.82783	0.137348	

Table 8. Effect of the compound of formula II (intravenous administration) on the reduction of blood pressure of cats

Sample, time	Animal 1 Systolic pressure (mmHg)	Animal 2 Systolic pressure (mmHg)	Animal 3 Systolic pressure (mmHg)	Animal 4 Systolic pressure (mmHg)	Average Systolic pressure (mmHg)	Standard derivation	T value	P value
Compound of formula II 5 mg/kg iv								
Before administration	226	183	211	178	199.5	22.86919		0.462627
10 min after administration	100	110	182	150	135.5	37.78448	0.027401	0.023299
20 min after administration	111	126	172	150	139.75	26.83747	0.014691	0.013666
30 min after administration	119	137	173	136	141.25	22.72114	0.01118	0.013315
40 min after administration	134	137	172	135	144.5	18.37571	0.009515	0.01859
50 min after administration	156	147	171	135	152.25	15.17399	0.013747	0.051646
60 min after administration	174	142	174	142	158	18.47521	0.030228	0.098648
90 min after administration	170	167	178	143	164.5	15.06652	0.043138	
120 min after administration	182	167	202	147	174.5	23.27373	0.176318	
150 min after administration	186	170	202	150	177	22.2411	0.208039	
180 min after administration	187	176	212	165	185	20.11633	0.377789	

	Animal 1 Diastolic pressure (mmHg)	Animal 2 Diastolic pressure (mmHg)	Animal 3 Diastolic pressure (mmHg)	Animal 4 Diastolic pressure (mmHg)	Average Diastolic pressure (mmHg)	Standard derivation	T value	P value
Before administration	169	138	163	115	146.25	24.78407		0.679085
10 min after administration	84	93	146	101	106	27.556	0.072856	0.038731
20 min after administration	79	109	137	98	105.75	24.24012	0.058123	0.024881
30 min after administration	80	111	136	98	106.25	23.55667	0.057871	0.052244
40 min after administration	100	111	134	97	110.5	16.78293	0.054118	0.047452
50 min after administration	117	120	134	97	117	15.25341	0.091131	0.116098
60 min after administration	131	120	130	92	118.25	18.19112	0.118371	0.139775
90 min after administration	122	129	144	92	121.75	21.85368	0.188616	
120 min after administration	123	129	148	96	124	21.49419	0.223773	
150 min after administration	124	140	148	98	127.5	22.05297	0.301473	
180 min after administration	123	148	156	108	133.75	22.18671	0.480745	

Table 9. Effect of the compound of formula Ib (oral administration) on the reduction of blood pressure of cats

Sample, time	Animal 1 Systolic pressure (mmHg)	Animal 2 Systolic pressure (mmHg)	Animal 3 Systolic pressure (mmHg)	Animal 4 Systolic pressure (mmHg)	Average Systolic pressure (mmHg)	Standard derivation	T value	P value
Compound of formula Ib 10 mg/kg o.s								
Before administration	150	209	185	237	195.25	36.89964		0.170789
10 min after administration	136	139	145	147	141.75	5.123475	0.028345	0.566023
20 min after administration	146	125	153	136	140	12.19289	0.029435	0.52967
30 min after administration	146	126	154	136	140.5	12.15182	0.03041	0.515824
40 min after administration	140	126	154	136	139	11.6046	0.027038	0.322939
50 min after administration	136	130	150	130	136.5	9.433981	0.021523	0.056753
60 min after administration	140	122	152	138	138	12.32883	0.025844	0.000905
90 min after administration	135	130	156	146	141.75	11.61536	0.032596	
120 min after administration	146	131	165	164	151.5	16.21727	0.072973	
150 min after administration	145	139	166	171	155.25	15.6285	0.092893	
180 min after administration	152	142	160	180	158.5	16.11418	0.117733	
210 min after administration			156	186	171	21.2132	0.452377	
240 min after								

administration			155	202	178.5	33.23402	0.619755	
	Animal 1 Diastolic pressure (mmHg)	Animal 2 Diastolic pressure (mmHg)	Animal 3 Diastolic pressure (mmHg)	Animal 4 Diastolic pressure (mmHg)	Average Diastolic pressure (mmHg)	Standard derivation	T value	P value
Before administration	134	156	116	154	140	18.8326		0.949541
10 min after administration	119	105	100	103	106.75	8.421203	0.018057	0.019637
20 min after administration	135	89	104	89	104.25	21.68525	0.047198	0.398951
30 min after administration	130	90	109	90	104.75	19.06786	0.039029	0.939613
40 min after administration	126	91	102	90	102.25	16.74067	0.024122	0.877248
50 min after administration	126	96	103	87	103	16.67333	0.02588	0.902185
60 min after administration	123	97	102	114	109	11.74734	0.031441	0.980271
90 min after administration	125	95	110	107	109.25	12.33896	0.034116	
120 min after administration	131	94	115	127	116.75	16.62077	0.113592	
150 min after administration	132	98	117	120	116.75	14.08013	0.09535	
180 min after administration	133	97	113	131	118.5	16.92139	0.14035	